NEURAL INTEGRATION IN LEARNING AND MEMORY: A HYPOTHESIS

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Federation of American Societies for Experimental Biology

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Despite the effort that has gone into establishing electrophysiological, anatomical, biochemical and behavioral correlates of learning and memory, thus far no clear basis has emerged. Many biochemical changes have been shown to accompany the neural functioning that proceeds as memories are "laid down," and many agents, physical and chemical, can disrupt the process. It is clear only that learning and memory require normal neural functioning and that interference with this functioning is variously effective

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depending on the timing and the severity of the disrupting input. "Switchboard " and "aggregate-field" hypotheses of neural integration may not be mutually exclusive. The former may represent rapid message-handling in "permanent" circuits, the latter may represent biochemical rhythms which reflect processes that may go to any degree of completion. The notion that the brain handles messages by "digital" versus "analog" techniques may also be a distinction artifically imposed by the techniques and nature of the instruments used to study these processes. The brain operates biochemically in time domains of about 10^{-3} to 10^{5} seconds, or even much longer if a lifetime is considered.

This report presents a hypothesis concerning neural integration in learning and memory based on biochemical events in this time domain. The hypothesis suggests that one of the events involved in intraneuronal message transfer may be pumping of lattice-vibrational states by signals pulsed to match the relaxation time of such states. The energy levels attainable can in turn match those required for conformational changes in macromolecules. Intracellular processes may play an equal or larger role in neural functioning than events measurable across the cell-membrane. The hypothesis provides a basis for study of early, time-variable concentrations of metabolic intermediates, metabolites and secretory products of neurons during memory and learning.

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Human Resources Research Office
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This research was supported by the Advanced Research Projects Agency of the Department of Defense and was monitored by the Air Force Office of Scientific Research under Contract No. F44620-74-C-0077 (ARPA Order No. 2808; Program Code 4D20).

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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in research in specific areas of biology and medicine. In addition, LSRO utilizes special consultants to prepare reports on specific topics where their expertise is applicable to particular needs for review and analysis.

This technical report was prepared for the Human Resources Research Office, Defense Advanced Research Projects Agency (DARPA), Department of Defense, under contract number F44620-74-C-0077 monitored by the Air Force Office of Scientific Research.

Under terms of the contract, LSRO agreed to assess recent developments in research on cellular mechanisms in learning and behavior because these have emerged as a central issue in neurobiology over the past two decades. Cellular processes at the synapses and protein metabolism within certain neural cells appear to be critical to learning, memory, and behavior. In the course of this study, LSRO staff had the opportunity to hear Dr. Sven A. Bach, M.D. present his hypothesis on biochemical synchrony of groups of neurons during learning and memory formation. Because the approach represents a novel concept of neural integration at the cellular level, Dr. Bach was asked to prepare a review of his hypothesis as a report to DARPA under terms of the LSRO contract.

This report was written by Dr. Bach who served as a special consultant to LSRO for this study. The report has been reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report has been approved and transmitted to DARPA by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

C. Jelleff Carr, Ph.D.
Director
Life Sciences Research Office

SUMMARY AND CONCLUSIONS ON PRESENT STATUS OF KNOWLEDGE

It seems that many degrees of persistence of memory can be demonstrated. Some memories are fleeting and delicate, others solidly ensconced and persistent.

Despite the immense effort that has gone into establishing electrophysiological, anatomical, biochemical and behavioral correlates of learning and memory, thus far no clear basis has emerged.

Very few workers today entertain seriously the idea of a memory trace laid down in the structure of specific molecules.

Many biochemical changes have been shown to accompany the neural functioning that proceeds as memories are laid down, and many agents, physical and chemical, can disrupt the process. It is clear only that learning and memory require normal neural functioning and that interference with this functioning is variously effective depending on the timing and the severity of the disrupting input.

The distinction between short-term and long-term memory may be an artificial one.

"Switchboard" and "aggregate-field" hypotheses of neural integration may not be mutually exclusive. The former may represent rapid message-handling in "permanent" circuits, the latter may represent biochemical rhythms which reflect processes that may go to any degree of completion.

The notion that the brain handles messages by "digital" versus "analog" techniques may also be a distinction artificially imposed by the techniques and nature of the instruments used to study these processes. The brain operates biochemically in time domains of about 10^{-3} to 10^{6} seconds, or even much longer if a lifetime is considered. Neural events occupy every portion of this spectrum.

One of the events involved in intraneuronal message transfer may be pumping of lattice-vibrational states by signals pulsed to match the relaxation times of such states. The energy levels attainable can in turn match those required for conformational changes in macromolecules.

Intracellular processes are poorly understood but may play an equal or larger role in neural functioning than events measurable across the cell membrane. Almost nothing is known of early, time-variable concentrations of metabolic intermediates, metabolites and secretory products of neurons during learning.

It may be impossible to dissociate learning from stimulation in any meaningful way. A wild rat is learning with every new experience. How this process may be biochemically related to the process of learning to prevent its feet from being shocked in a Skinner box may be difficult to discover.

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I. INTRODUCTION

With the current state of knowledge it is impossible to present a complete hypothesis of the integrative neural processes for the acquisition, storing and retrieval of memories. However, when one studies the various current views of these events, there remains the nagging belief that there must be some unifying principle which could bring together the diverse opinions represented by the "cellular-connection" or "switchboard-model" theories of neural integration on the one hand and the "aggregate field" hypotheses on the other (John, 1972; Kandel and Spencer, 1968; Sommerhof, 1974). These models are not necessarily mutually exclusive, but simply focus on different properties of the nervous system. While no such principle is proposed, an examination of some of the experimental data and the models of neuronal function in a temporal framework might aid in constructing a future scheme for unifying current hypotheses.

As indicated by the title, the emphasis is on learning and memory, by which is meant the acquiring by the animal organism of new behavioral patterns appropriate to new situations and their retention and recall as evidenced by appropriate behavior when again faced with the same, an analogous, or a related set of stimuli.

The dynamic features of this process will be stressed and the described events placed into their particular time domains. This will help to correlate biochemical, electrophysiological, behavioral and anatomical changes in such a way that their interrelationships can be sorted out on a reasonable causal basis. Whatever may be the theoretical views of neural events in learning, they must all be possible within the stated times and match the observed behavior.

This report is not intended to be an exhaustive review. Many excellent ones on different aspects are available and are indicated in the bibliography. The discussion is limited to well-documented experimental results which will be fitted into an interpretation of memory as a dynamic process involving many different hierarchical levels from molecules to the whole organism. Memory is to be viewed as a set of biological events highly specific in sequence, both in space and time.

II. OBSERVATIONS ON MAN

To form a memory, a stimulus (or group of stimuli) must be perceived. The beginning of a memory must coincide with the beginning of perception. There is considerable evidence that regardless of physical mode of the stimulus, its perception by the human begins about 70 milliseconds after its onset (Efron, 1967; Gibbon and Rutschmann, 1969).

There seems to be a general agreement that there are at least two kinds of memory in man, a short-term and a long-term one. The former may decay over a period of seconds to hours, and the latter, after "consolidating" over a number of hours, days or even years may persist in a more or less permanently available "store." Experimental and clinical evidence in man indicates, however, a complete gamut in time of the memory store. Thus, very short-term memory, decaying in a few seconds has been documented (Lunney, 1974; Wingfield and Byrnes, 1972). In patients with severe recent memory deficit following bilateral hippocampal lesions, retention of three-figure numbers or pairs of unrelated words was possible for several minutes, provided the patient's attention was not diverted from the task. However, these patients suffered a major, permanent inability to learn anything new (Scoville and Milner, 1957; Smythies, 1970a). There is some evidence, though, that nonverbal tasks might still be learnable (Ervin and Anders, 1970).

Extensive lesions involving one cerebral hemisphere before or during childhood development can be removed by extirpation of the entire hemisphere with surprisingly few serious effects on either verbal or non-verbal learning. In an adult with already established skills, a lesion which involves the brain subsequent to their acquisition is a more serious matter and shows the effect of specialization, the left side predominating as to language skills, and the right as to visual ideational, non-verbal reasoning and constructional skills. Considerable variability exists in the degree of such specialization (Burklund, 1972; Smith, 1972).

Patients subjected to electroconvulsive therapy (ECT) lose their ability to recognize one-season TV series up to 3 years before treatment, but programs aired before that time (4 to 17 years) are recalled just as well as by controls. The equally well-remembered material decays from then on at the same rate in both groups (Squire, 1975). It thus appears that consolidation for this sort of material takes place over several years. Epileptogenic foci in the temporal lobe often evoke an aura of very complex and highly vivid content which reconstructs past experience in detail. Direct stimulation of this region (the lateral, anterior and inferior temporal cortex) during surgery can reproduce the memory. Often the remembered episode

unfolds in time as stimulation is continued. The patient reports that even though the recollection is clear and vivid, it is recognized as extraneous to the immediate surroundings with which he remains fully in contact during the procedure (Penfield, 1958).

Language production (which must of necessity involve recall) is preceded by slow,negative potentials recorded at their maximum over the foot of the left frontal convolution of the cerebral cortex (Broca's area). These potentials precede the articulation of the words by about one second ("readiness potential"). This is before the actual "voice trigger" which is a large positive potential accompanying the actual vocalization (McAdam and Whitaker, 1971).

Although scalp electroencephalograms often seem to have the character of random noise, there is general agreement that definite rhythms can be observed and are consistent enough to be classified and studied.* Autocorrelation and spectral analyses have revealed many subtleties in the frequency components (Thompson and Patterson, 1974). Highly pertinent to the present paper is the study of an adult human whose "idling" electroencephalographic (EEG) pattern was Gaussian 66 percent of the time. During performance of a mental arithmetic task, the portion of the record having a Gaussian character dropped to half of that percentage, implying an increase in the cooperative activity of cortical neuronal elements during the performance of the task (Elul, 1969). Many other electroencephalographic correlates of human behavior and stimulus perception have been recorded (Gasthaut, 1957; Regan, 1975; Ritter and Vaughan, 1969; Rosenfeld *et al.*, 1969; Tecce and Scheff, 1969) to list only a few.

Human memory must then be thought of as a process (or processes) which can be initiated within about 70 milliseconds of the onset of a stimulus, and then last for seconds, minutes, hours or years depending on how the nervous system responds.

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*In 1958 I observed but did not publish the fact that electrodes placed in a bowl of Jello (cherry) will produce a great variety of signals superficially resembling an EEG. "Sleep spindles" can be produced by walking heavily on the floor near the preparation. The normal activity of chimpanzees in an adjacent area was sufficient to induce a random set of signals easily recorded.

III. EXPERIMENTAL MODELS: BRAIN STRUCTURE AND ORGANIZATION

Mammalian studies relating brain function and learning form a vast literature. Only a few of the structures implicated and enough of the synaptic organization and function are reviewed to highlight the temporal relationships.

Neurons are connected by every conceivable combination of junction, varying from several types of membrane juxtaposition to synapses operating through chemical transmitters. These may be axodendritic, axosomatic, axoaxonic of dendrodendritic and arranged singly, reciprocally or serially. The synaptic vesicles which contain the transmitter substances are either small (20 to 40 nm in diameter) or medium sized (50 to 90 nm), the former probably associated with acetylcholine and certain amino acid transmitters, the latter with norephinephrine (DeFeudis, 1975; Shepherd, 1974a). A typical synaptic cleft could be represented by a flat cylinder of 1 μm radius, 50 nm thick, and therefore with a volume of 1.6×10^{-16} liters, which provides room for about six H_3O^+ ions at a pH of 7.2.

The total number of neurons in the human brain is often said to be 10^{10} (Griffith, 1971). However, the number of granule cells in the cerebellum alone is four times this. The degree of convergence of inputs upon single neurons can be very great. Purkinje cells in the cerebellum for example have more than 100,000 dendritic spines, each connecting with an individual granule cell (Shepherd, 1974b; 1974c).

Action potentials, typically pulsed signals in the millisecond range, of 50 to 150 mv are the presynaptic signal traveling down an axon at rates of one to one hundred meters per second. Such impulses are associated with an inward sodium current and a slightly delayed and more prolonged outward potassium current (Hodgkin and Huxley, 1952). The Na^+ and K^+ exchange is supposed to be restored by ionic pumps. The initial event, the alteration in conductance to the two ions, has been consigned thus to the membrane. Just how the membrane does this is a puzzle. Some cells spontaneously generate impulses (the pacemaker potential). This intrinsic rhythm is supposed to be a property of the membrane, but is little understood (Shepherd, 1974d). These signals may be considered part of a pulse-coded system and are characteristic of many regions of the nervous system. They are often used as models of information transfer by the cyberneticists and are susceptible to highly sophisticated mathematical treatments (Griffith, 1971; Wiener, 1948a).

In contrast to the digitally coded responses just mentioned, other areas process information as analogs. This is true of the retina, which performs the ultimate feat by converting an extremely high-frequency signal (6×10^{14} Hz for 500 nm light) to a signal usable by the brain.

Many of the brain neurons in regions important to memory show responses which would be called "ringing" in an amplifier. That is, the postsynaptic response to a presynaptic pulse is characterized by a rapidly decaying train of oscillations. Thus a pulse to a thalamic sensory relay neuron elicits a train at about 300/second (Shepherd, 1974e). In a hippocampal pyramidal cell high-frequency bursts are seen up to 400/second (2 to 4 spikes) and moderate duration trains of 5 to 8 spikes at about 100/second, which appear to decay exponentially (Kandel and Spencer, 1961). In the neocortex a similar pattern can be seen (Shepherd, 1974f).

In contrast to the self-maintained nerve impulse which reaches its destination undiminished, there are electrotonic dendritic potentials which spread passively. These have been elegantly analyzed (Rall, 1970; Rall and Rinzel, 1973). These can be treated by so-called cable equations either as steady states if slow enough, or as transients if the time constants of the neuronal membrane are in the same time domain as the events. The spread of electrotonus through a highly branching tree (or a converging one) can form the basis for an intricate network of conditional probabilities with a very high information content (MacKay, 1973). If this idea is coupled with the ones to follow on synchronous activity in a very wide temporal range of oscillatory activity, there is a more than adequate basis for all of the interrelationships required for acquisition, storage, and retrieval of highly complex behavioral patterns. The basic point here to be stressed is that neurons are tied together by signals which can be very fast (millisecond) or so slow as to take on the character of steady-state potentials. This entire gamut has been observed and recorded in the human brain.

Much of the integrative activity of the brain is believed to be carried out in the limbic system. Within the limbic system, the hippocampi display a remarkable plasticity in recorded responses and in their rhythmic potentials. In carnivores and lagomorphs a prominent 5 to 7 Hz (theta) rhythm is manifest. Human hippocampal activity shows faster components. This rhythm is said to be imposed through a path involving hypothalamus, septum and fornix by reticular formation activation. This imposition of theta rhythm has been variously viewed as an inhibition of the hippocampus (Smythies, 1970b), a nonspecific response indicating general alertness and muscular activity (Klemm, 1972), or a temporal correlate of memory storage processes (Landfield *et al.*, 1972).

In the rat, afferent stimulation via the septum produces postsynaptic potentials and firing in hippocampal pyramidal cells accompanied by large,

standing field potentials, negative at the surface down to the pyramidal cell layer, and positive deeper (apical dendritic area). Repetitive stimulation leads to an evoked response which is constant at slow stimulation rates (<1/second) but shows facilitation at higher rates (4 to 10/second) so the amplitude increases. Faster rates yet produce inhibition. Continued stimulation at the optimum rates induces seizures (Izquierdo, 1972).

Stimulation by 10/second, 0.2 msec., 0.2 mA pulses in the locus coeruleus (a pontine nucleus) of the rat results in inhibition of spontaneous firing in hippocampal pyramidal cells. The response is delayed by 150 to 200 msec. after stimulus onset and lasts 5 to 120 seconds after termination of stimulus. The iontophoretic application directly to the cell of norephinephrine results in inhibition with a similar long latency and persistence. Other ancillary pharmacological tests make it quite convincingly apparent that this pathway is mediated by norepinephrine terminals (Segal and Bloom, 1974a; 1974b).

In rabbits, tetanic stimulation of the perforant path evokes a monosynaptically transmitted response in the dentate cortex of the hippocampal region. This is a population response of granule cells and is characterized by synaptic potentiation which lasts from an hour up to several weeks. This potentiation appears to be due to both increased synaptic efficiency between the perforant path and granule cells, and an increased excitability of the granule cells (Bliss, 1973).

From these data it appears that this portion of the limbic system exhibits a good deal of electrophysiologically demonstrable plasticity and a great variety of time domains from several milliseconds up to hours and even weeks.

IV. ANATOMICAL PLASTICITY IN THE NERVOUS SYSTEM

Implicit in a "switchboard" theory of memory is the establishment of new connections or at least their facilitation. This is a necessary condition for learning paradigms involving conditioned and unconditioned stimuli. Whether all of such facilitation can be laid to changes at the synapse itself is another question. It may well be that intracellular processes at least share in this.

The brain exhibits modifiability in embryonic and early life. ". . . the processes concerned, however, remain among the most profound unsolved problems of biology" (Shepherd, 1974g). It appears that early in development neurons may be able to connect in a wide variety of ways, that is, if moved to a new location in the embryo they will form connections appropriate to that location. Later on they lose this ability and become "uniquely specified" (Jacobsen, 1969). The sensory richness of the environment seems to be important in the developing brain. In a number of experiments, rats were raised in three different environments: singly caged, in empty cages with 2 or 3 animals per cage and in "enriched" environments -- 10 to 12 animals per cage with toys. The degree of complexity in the environment correlated with changes in enzymatic activity, depth of cerebral cortex and increases in both wet and dry weights of cerebral cortex (Bennett *et al.*, 1969; Diamond *et al.*, 1964). Rats from the enriched environment exhibited superior learning behavior. Pyramidal neurons from occipital cortex in these rats showed an increase in the amount of dendritic material per neuron, which was due to increased branching within the original, or only slightly greater, volume. Since the dendritic spine frequency on these branches was equal to or greater in "enriched" rats than in "impoverished" rats, the number of dendritic synapses per neuron was clearly higher in the former. These changes in the developing rat were in basal dendrites. When adult rats were given a set of visual discrimination tasks over a 35-day period an increased branching was observed in outer apical dendrites but not in the basal region. Quantitatively, these effects were much smaller than those seen in the developing brain (Greenough, 1975).

Cats raised from birth with one eye viewing horizontal lines and the other viewing vertical lines showed elongations in the direction of the cortical receptive fields corresponding to the original restricted input (Hirsch and Spinelli, 1970).

Humans with astigmatism showed a difference in resolution sensitivities to lines oriented with or across the cylindrical axis of the optical error even though an optical correction had been introduced. This tends to indicate a neural lack analogous to the results of the feline experiments (Freeman *et al.*, 1972).

Newborn rats subjected to a battery of stimuli for 20 to 30 minutes per day and then killed on the 8th day showed increased numbers of dendritic spines per unit length of cortical pyramidal dendrites without apparent change in cell body dimensions or in apical or lateral dendrite length. This increase was most apparent in the basilar and oblique branches. The rapid Golgi technique stained more cells per section of the brains of stimulated rats. The authors, significantly, suggest that the cells accepting the stain may be those that are functionally involved at the time of tissue preparation (Schapiro and Vukovitch, 1970).

Anatomical evidence for plasticity in the adult animal is meager (Raisman, 1969; 1973; Shepherd, 1974g). The first author has described repair processes in septal nuclei of rats which indicate that establishment of new synapses is on a heterosynaptic basis, "a re-occupation of de-afferented sites by local intact terminals." He cautions that these experiments do not provide any direct evidence for rearrangement of synaptic connections as a result of the learning process, only that such rearrangements are possible in the adult brain.

In an excellent review of the morphological correlates of function, Berry *et al.* (1973) states that if such changes are to play a role in learning and memory the "time constant" must be related to, and be compatible with consolidation of the trace. He noted that changes in retinal synapses after exposure to light have occurred within 3 minutes, while darkness-induced changes are demonstrable after 24 hours. He lists the factors to be considered in assessing the various time constants as "the site of manufacture of proteins, lipids, etc., the distance between this site of synthesis and the site of structural change, and the speed at which metabolites can reach this site and be incorporated into new membranes. . ." To this one should add the necessity of considering periodic fluctuations in the concentrations and activities of the cellular components.

The anatomical evidence for plasticity in the nervous system is based for the most part on studies of the developing brain. There might be some anatomically demonstrable effect of learning in the adult, but so far the evidence appears to be difficult to interpret. Plasticity as a feature of the learning process in the adult brain may well be demonstrable only on a functional level of investigation, be it electrophysiological, biochemical or behavioral. What is certain is that the temporal aspects of all of these measurements must be carefully considered.

V. BIOCHEMISTRY AND CHEMICAL EFFECTS

There is a vast literature on biochemical changes resulting from training and the effect of various chemicals on retention of learned behavior. Much of the evidence is difficult to interpret because of the problem in sorting out the effects of stimulation and the specific learning correlates. This may be inherently an insoluble problem because surely all learning involves stimulation of groups of neurons in a well-defined sequence. It would nonetheless be significant if demonstrable intraneuronal biochemical changes were shown to result from neuronal activity during learning.

The biochemistry of learning and memory has been competently and thoroughly reviewed (e.g., Glassman, 1969; Glassman and Wilson, 1973; and Rahwan, 1971). In addition several compilations of review-type articles exist (Albers *et al.*, 1972; Ansell and Bradley, 1973; and Essman and Nakajima, 1973).

Many of the reported results have to do with suppression of protein synthesis by drugs such as puromycin, cycloheximide and acetocycloheximide. All have caused amnesia in various species, but side effects are difficult to isolate from the memory effect. Reducing the synthetic rate of any essential molecule may impair memory. The problem is in locating the mechanism specific for memory formation (Dunn and Bondy, 1974). There may indeed be no such specific mechanism.

Actinomycin-D which inhibits DNA-dependent RNA synthesis has often been used to study memory storage. It has been supposed that this action is the reason the agent suppresses long-term memory storage. However, low doses injected bitemporally in mice have little effect on cerebral RNA synthesis although they do produce cellular damage and electrical abnormalities in the hippocampi as well as interference with long-term memory. These injections were effective if made 3 hours before and up to 24 hours after training. These effects are believed to result from toxicity to the hippocampi rather than inhibition of protein synthesis (Squire and Barondes, 1970).

Cycloheximide which is quite toxic in mice and anisoheximide which is relatively nontoxic at doses inhibiting protein synthesis to the same degree in mice, both affect long term memory, but the former is more effective. The anamnestic effect is related to the duration of inhibition of protein synthesis (Flood *et al.*, 1973). Cycloheximide apparently does not act through its effect on general activity: initial hyperactivity within 3 minutes minutes, followed by a return to normal in 30 to 40 minutes, then decreased activity (Segal *et al.*, 1971).

Some of the most elegant work on this subject has been done by Hyden (1973) His extensive investigations have involved microdissection in which neuronal and glial elements are separated. This author stresses temporal aspects of synthesis of protein in the brain, the mutual interrelationship of neurons and glia, a change in base ratios of RNA and the formation of brain specific proteins, one largely glial (S-100) and the other neuronal, during the process of learning in animals. He found that nerve cells within the limbic system increase protein synthesis at the beginning of training, this correlating well with electrical activity described by other authors. He found neocortical protein synthesis to be inhibited while it was stimulated in hippocampus. The hippocampal S-100 protein was found to separate into 2 fractions in trained animals (as opposed to a single electrophoretic fraction in controls). One fraction was associated with excess calcium, an ion which can cause conformational changes in that protein. He regards the selective uptake of calcium in neurons as having a dual function -- induction of a conformational change in a membrane protein (S-100) and increased excitability -- which "could be the mechanism of translating electrical activity into remaining macromolecular patterns and could constitute the identification mechanism." He suggests that neurons sharing protein patterns of their synaptic membranes would respond to the "same signals." Whether or not signals of frequency appropriate to conformational change in macromolecules could be transmitted at a distance is a debatable matter. However, immunologic and enzymatic reactions can be carried out at solid-liquid interfaces when a 5 to 20 nm formvar membrane separates the molecules involved if the interaction takes place on an orienting surface. The interaction is via specific long-range Van der Waals orienting forces (Rothen, 1973).

There is obviously no likelihood that oscillators at such frequencies could be linked via nerve impulses. However, events at both the pre- and post-synaptic membranes could be mediated thus between oriented molecules without their actual contact.

There is a possible mechanism for inducing conformational change in macromolecules via pulsed electrical signals in the frequency ranges encountered in the nervous system. Von Foerster (1969) has made the suggestion that pumping of lattice vibrational states could occur in macromolecular crystals as the result of trains of pulses of 50 to 180 mv at pulse rates corresponding to the average lifetimes of such states. This concept deserves a closer look. Von Foerster's development of this notion is based on his consideration of a molecule as a basic computer element. Even if one considers such a system only as a membrane element (say in the presynaptic membrane) and not as an information storage device *per se*, the

energetics of the system are extremely interesting. The average lifetime of a configurational state can be represented by $t=t_0 \exp(\Delta E/RT)$; t_0 for the states in question is of the order of 3×10^{-15} second which is associated with electron orbits within the crystal. ΔE, the height of the "energy trough" for enzymes and other proteins is around 28 k cal/mole or 1.2 electron-volts per molecule. At normal body temperature 310° K, t works out to be 10^4 to 10^5 seconds, the average lifetime of a state before it changes through quantum-mechanical tunneling. That is, 3 hours to about 1 day for the lifetimes of these conformational states.

Another intrinsic set of oscillations is that associated with lattice-vibrational states in macromolecules. These are of the order of 10^{-4} seconds ($t_0=10^{-4}$ second). ΔE's are of the order of 50 to 180 mv, which are in the domain of ordinary action potentials. Furthermore with volleys of impulses separated by intervals corresponding to average lifetimes of these states, pumping could bring the total energy level up to that required for a change in configuration. Not to be taken too literally, but as representative figures based on $t_0=10^{-4}$ sec, we see that at:

$\Delta E = 50$ mv $t = 6.5 \times 10^{-4}$ sec
$\Delta E = 100$ mv $t = 4.2 \times 10^{-3}$ sec
$\Delta E = 180$ mv $t = 8.4 \times 10^{-2}$ sec

Pulse rates matching the average lifetimes of these states are 1540, 240, and 12 per second, well within the range of observed signals in the brain. Pumping by trains of such pulses to bring ΔE up to 1.2 electron volts per molecule would have to last for 0.016, 0.05, and 0.55 seconds respectively. (By way of reference H-bonds in water have energies of the order of 0.3 electron volts per bond, 6 to 7 k cal/mole.) This sort of interaction may play an important role especially in the presynaptic region. The role here of vicinal water, highly organized as it is, may be critical (Drost-Hansen, 1971; 1973). Onsager (1969) has observed that ice may be a protonic semiconductor device; vicinal water may act in much the same way.

VI. OSCILLATIONS AND RHYTHMS IN BIOLOGICAL SYSTEMS

Wiener (1948b) has treated theoretically the concept of feedback control of biological systems. In particular he has considered systems of nonlinear oscillators which are characterized by "a discrete set of amplitudes for which the system will oscillate at a given frequency as well as a discrete set of frequencies for which the system will oscillate."

Feedback control in the cell is performed generally through the actions of regulatory (allosteric) enzymes (Monod *et al.*, 1965) which exercise their control at critical points in synthetic chains through repression of enzyme synthesis and end-product inhibition of enzyme activity (Datta, 1969). The dynamics of this type of control are such that there is necessarily a continuous oscillatory activity in cellular constituents. These oscillations manifest themselves in various rhythms (Goodwin, 1967). Goodwin (1963) in a remarkable book, Temporal Organization in Cells, has treated this subject in great detail. His mathematical treatment of fast metabolic chains as well as the slower macromolecular syntheses is made possible by considering the slow components as constants or as slowly changing parameters in faster systems. The order of magnitudes of the relaxation times derived are of interest. For diffuse interactions and transfer of "small molecules" they are about 0.01 to 0.1 second. Turnover rates of substrates of intermediary metabolism are about 10 to 10^4 seconds. As an example, glucose 6-phosphate in ascites cells can increase from 0.05 to 0.8 mM/g within a minute. These "metabolic oscillators" if coupled by reciprocal feedback in linked chains might have longer periods associated with them, even up to 5 to 30 minutes. The "epigenetic" systems, that is, synthesis, diffusion and interaction of macromolecules, are rather fast in bacteria e.g., 5 seconds to synthesize a single protein molecule. This process requires minutes in the higher organisms. RNA synthesis takes one second in bacteria and about a minute in eucaryotic cells. For enzyme induction in bacteria a typical case is the 4-minute time-lag to beginning beta-galactosidase activity in E. coli after beta-galactoside is added to the culture. The rat liver on the other hand requires 2 hours before tryptophane pyrrolase synthesis begins after intravenous administration of tryptophane. Overall the time constants for epigenetic systems are 10^2 to 10^4 seconds (1.5 minutes to about 3 hours). A metabolically active protein could have an associated relaxation time of 10 to 20 hours. Subharmonics of such periods could lead to even longer times. In his later paper (Goodwin, 1967) the author analyzes a simple feedback repression circuit in growing cells. There is no reason that this type of analysis would be valid only for growing cells; it is merely a convenient model. Goodwin ends his paper with a very significant set of statements in which he relates these concepts to specificity of neural connection during development by way of a sequential chemical code based on intrinsic rhythms

in size of metabolic pools in which the specificity is high indeed yet requires no involvement of genes at all. Gander (1967) has described a model of an oscillatory system containing allosteric enzymes and stresses the concept that such a system could serve as a pacemaker for other coupled systems. He suggests a view of aging based upon synthesis of aberrant allosteric enzymes, and also presents some general classes of experiments that could be devised on the model.

Barondes (1965) has suggested that neuronal plasticity might usefully be considered as a manifestation of biochemical regulatory processes analogous to those found in bacteria. The type of feedback inhibition under consideration depends on conformational changes in existing proteins rather than changes in RNA or protein synthesis (Atkinson, 1966). However, Mitchison (1969) reviewing patterns of enzyme synthesis in synchronous bacterial cultures finds evidence for an orderly sequence of transcription throughout the cell cycle, i. e. a genetically controlled sequence. These findings, though, may not be applicable to the nondividing neuron.

It appears that within the cell there is an adequate biochemical basis for generation of rhythmic activity of every conceivable form and with time constants from thousandths of a second up to hours or days. Entrainment of such rhythms between adjacent cells and between distant cells can be readily imagined. It may well be that much of the rhythmic activity in the brain is based on intracellular systems of oscillating pools of metabolites, intermediates and other products of branched synthetic chains which are coordinated through electrical and humoral signals. These oscillations may have characteristic periods of fractions of a second up to hours or days, perhaps even longer.

VII. ENERGY EXPENDITURE IN THE BRAIN

It is a remarkable fact that the brain which makes up only 2 percent of the body's mass (1400 g out of 70 kg) uses 20 percent of the oxygen required and about the same proportion of the total energy (Dunn and Bondy, 1974). The rate of energy production (about 20 watts) is very constant. It seems to make little difference what the brain is doing; daydreaming, sleeping, or intense mental activity all require the same power. A large proportion of this energy consumption has been ascribed to maintenance of ionic concentration gradients across active membranes through ionic pumps. If the sodium pump alone takes 10 to 20 percent of the total energy metabolized (Hazlewood, 1972), it seems an unusual feat for an intramembrane system confined to what surely is a small part of the entire cell mass to expend this high proportion of energy. The power to mass ratio must be enormous. The purpose here is not to enter the "membrane" versus "association-induction" controversy (Ling and Cope, 1969) but rather to direct attention to the inside of the cells with their numerous metabolic compartments which form, in aggregate, multicellular compartments. These latter show a constant average pool size which cannot reflect the endogenous fluctuations in single cells acting randomly, but could show such fluctuations when the cells are synchronized with respect to the particular pool involved.

The biochemical implications of this view are significant. If, for example, an increase in concentration of a particular molecular species is found in a specific region of the brain at, say, one hour after a learning experience, the increase may reflect not a real rise in level of that species but merely a synchrony between cells of fluctuations with a long time-constant. Thus a sampling over sufficient time, if it had been possible, would have revealed a long, slow rise and fall with an average level exactly the same as the level found when the cell population was acting randomly.

This mechanism may explain many of the experimental results that have been reported in which early samples show increases in RNA, while samples taken later show no difference or even a decrease. This picture of brain activity would also account for the remarkably constant energy expenditure of the brain because synchronous activity *per se* need not raise the average metabolic requirements to any great degree. This may then be the reason why chemical samples which, like the Golgi stain, are "snapshots" of neural events, reveal great swings in levels of concentration depending on their timing in relationship to the particular neural activity that is in progress at the moment of tissue death.

VIII. AN OUTLINE HYPOTHETICAL VIEW

Stimuli from the environment are transduced by receptor organs into signals of every conceivable waveform and time dependence. It is supposed that regardless of mode of transfer between neurons, the cells can respond to a wide variety of signals because of the membrane and intracellular branched and cross-linked biosynthetic chains controlled by regulatory enzymes. These may serve to entrain particular rhythms with time constants of milliseconds to hours or days. A sequentially coded set of signals, for example, those produced by the reticular formation during arousal, coupled with the input from the periphery may entrain a sequence of similar nature in widely separated groups of neurons. The biochemical substrate of neural integration would thus consist of oscillating multicellular metabolic pools which are links in a metabolic chain. The degree to which syntheses progress would determine how long the "statistical configuration" or "coherent pattern of discharge of neurons" lasts (John, 1972). The process could continue to completion of new protein molecules and new structures at or near synapses, or decay with time over seconds to years.

Perhaps the process itself as much as the products of the process holds the essence of neural integration. It may be that many of the biochemical syntheses in the neuron have no other function than the evanescent waxing and waning of concentrations of intermediate molecular species. The gradual bringing into biochemical synchrony, a synchrony in a stochastic sense only, of large groups of neurons may be the main early events in learning and memory consolidation. Failure to reinforce this process may lead to gradual desynchronization and reestablishment of random biochemical activity. As training progresses, more and more of the switchboard element might come to the fore and what was originally a loosely coupled set of rhythms involving many neurons might become more finely tuned and involve fewer cells. These would be more tightly coupled by actual anatomic changes in synapses and in subsynaptic intracellular elements located largely in the dendritic trees. The membrane and intracellular elements may be pockets of potential biochemical activity to be set in motion when signals suitably coded in time trigger them into sequential activity.

IX. SUGGESTIONS FOR FUTURE RESEARCH

Further development of this hypothesis will require assembly of detailed, reliable information on electrophysiological, biochemical, histological and behavioral aspects of learning and memory. The choice of inclusion of supportive reference documents would rest on whether or not careful timing of the events and the measurements had been performed.

There would be little to gain from biochemical examination of gross tissue samples. It is unlikely that new species, or an unusual concentration of molecular species would be observable in such samples. Effort should rather be directed to the temporal pattern of syntheses in specific types of neurons. A common sequence of rise and fall in concentrations of various molecules might reveal which brain regions were in biochemical synchrony, thus extending to slower components the electrophysiological observations (such as on evoked potentials) now being made.

It may be that electrophysiological observations can reveal only the patterns already fixed and that synchronous activity associated with apparently random electrical patterns can be sorted out only on a much slower time scale. Perhaps this scale is accessible more to biochemical than to electrophysiological techniques.

At the fast end of the time spectrum of neural activities, some of the early biochemical events may be accessible to relaxation techniques down to sub-millisecond intervals (Winkler, 1974). Immunofluorescent studies could reveal biochemical synchronies if carried out on many different brain regions in such a way as to ensure simultaneity of sampling and adequate temporal correlation of the behavioral aspects.

Understanding of events at the synaptic cleft is still meager. There is no doubt that in the 50 nanometer space the water is highly organized and cannot be considered in any sense as resembling bulk water in its properties. Proton jumps, "diffusion" of metallic ions, the degree of incorporation of transmitter molecules into the organized space, and action at a distance through this medium all require much more understanding of water at an interface. Discrete changes in structure with temperature and their possible persistence in metastable configurations might be exploited to further such understanding.

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